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**Assessment of Salmonella Species and Escherichia Coli Prevalence, as well as Antimicrobial Profiles, in Sliced Pineapple Vended within Abakaliki Metropolis**

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**ABSTRACT**

The prevalent practice of vending sliced pineapple and various fruits on the streets of Nigeria due to cost constraints among buyers has raised concerns regarding potential contaminations and subsequent health risks to consumers. This study procured sixty samples of sliced, ready-to-eat pineapple from street vendors in Abakaliki Metropolis, analyzing them for Salmonella and E. coli contamination using microbiological and biochemical techniques. The isolates underwent antibiotic sensitivity screening via the disc diffusion method. The findings revealed a 20% prevalence of Salmonella species and a striking 70% prevalence of Escherichia coli in the sliced pineapple fruits. Further investigation into antibiotic susceptibility exhibited a concerning pattern of high multi-drug resistance among the isolated organisms. Salmonella species showcased resistance percentages ranging from 50% to 100% against ceftriaxone, nitrofurantoin, meropenem, pefloxacin, chloramphenicol, ofloxacin, and amoxicillin. In comparison, Escherichia coli exhibited resistance values varying between 28.6% and 100% against the same antibiotics. This study emphasizes that the prevalent practice of slicing and vending fruits on the streets significantly contributes to the dissemination of multi-drug resistant pathogens. Urgent intervention and discouragement of this practice by pertinent authorities are imperative to mitigate the escalating health risks associated with such contaminated fruit vending practices.

**Keywords:** *Street vended fruits, sliced pineapple fruits, fruit contamination, multi-drug resistance, Samonella species, E. coli.*

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**INTRODUCTION**

Pineapple (*Ananas comosus*) is an essential fruit that is valued by many people due to its enormous health benefits. It is peeled and sold in many markets and road sides for easy accessibility [1-3]. According to [4] pineapple is a cylindrical false fruit (pseudo-fruit) of the family Bromeliaceae and consists of a thickened, fleshy, very juicy axis core and inedible, scaly, warty skin, resembling a pine core. Only the polygonal, flattened outsides of the individual fruits are visible at the surface of the multiple fruit (syncarp). Pineapple can be used for preparation of jellies, juice, jams and fruit salad. They contain high amounts of sugars and other nutrients and low in pH which makes it

particularly prone to bacteria infestation [5-6]. Because of the softness and high content of nutrients (carbohydrates, proteins and fats) in ripe fruits including pineapples, they become susceptible to infestation by a variety of disease-causing organisms including bacteria [7-9].

According to [10], sliced fruits are fruits that have been cut open and sliced into bits, but remain in its fresh state and displayed for sale and for consumption. In other words, , fruits that can be directly purchased from street vendors or hawkers or at local markets and consumed instantly without further processing as they have already been prepared by the sellers are generally called ready-to-eat fruits [11-13]. Such fruits are directly purchased from the roadside vendors or hawkers or at local market without necessarily having to undergo any further treatment before consumption [14-17]. The high vitamins (such as vitamins B, C, K), minerals (calcium, potassium, magnesium) and fibre contents in fresh fruits and vegetables play vital roles in human nutrition [18-21]. They provide a healthy and balanced diet and can prevent chronic diseases such as heart diseases, cancer, diabetics, and obesity including several micronutrient deficiencies especially in developing countries [22-24].

Few years ago, the consumption of sliced/ready to eat fruits (such as pineapple (*Ananas comosus*); watermelon (*Citrullus lanatus*) and pawpaw (*Carica papaya*)) in Nigeria have increased significantly because of ease of access, convenience, nutrition and most importantly cheaper than the whole fruits [25-27]. Cross contamination of street-vended fruits (pineapple) is increased by possible unsanitary processing and preservation methods, slicing with dirty knives, as well as the open display of the ready-to-eat fruits which promotes spontaneous visits by flies and dust. Other major sources of infection in sliced fruits may be the washing water used by the vendors who peel the fruits and sell to final consumers, if washed. It could also occur during the growing season, harvesting, handling, transport, post-harvest processing and marketing conditions or after purchase by the consumer [28-31].

In Nigeria, selling handy ready-to-eat sliced fruit and vegetables in the streets has since become a widespread and flourishing business [32]. Such is sold by unlicensed street vendors with poor educational background and lack of training in food hygiene [33]. Increased consumption of vended fruits, coupled with the associated risk of disease to which consumers may be exposed to, is a matter of great concern. Microbiological studies from many developing countries, carried out on foods sold in the street have shown high bacteria counts. Enteric pathogens such as *E. coli* and *Salmonella* spp are among the greatest concerns during food related outbreaks [34]. Several bacteria such as *Salmonella* species, *Shigella* species, *Listeria monocytogens*, *Campylobacter* species, *Aeromonas* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* species have been associated with contaminated sliced fruits [35-37].

In recent decades, there has been an increase in the prevalence of antimicrobial resistance among food pathogens [38-41] and the rise in the occurrence of antimicrobial resistance bacteria in humans, animals and the environment is a major concern in both human and veterinary medicine [10]. Antibiotic resistant organisms can contaminate fruits and vegetables through several sources including contaminated water used for irrigation, animal wastes used as manure and the nature of the cultivation sites. For instance, manure used in enhancing soil fertility harbors bacteria that can carry Antibiotic Resistant Genes (ARG), which can be horizontally transferred to soil bacteria and can in turn contaminate fruits and vegetables [3]. This is also applicable when there is faecal contamination of soil or spray of soil with antimicrobial agents [2]. This work therefore aimed at determining the presence and prevalence of *E. coli* and *Salmonella* species from sliced pineapple fruits together and assessment of their susceptibility to commonly used antibiotics.



**Plate 1:** Photo of a Typical Fruit Vendor Shop in Abakaliki, Nigeria.

## **MATERIALS AND METHOD**

### **Sample collection**

A total of 60 sliced pineapple samples were bought from different selling points into sterile polythene bags, and were transported to Applied Microbiology laboratory section of Ebonyi State University, Abakaliki for analysis.

### **Isolation of bacteria**

A sterile swab stick each was used to swab the surface of each sample collected. Each swab stick was used to inoculate a test tube containing 5 ml of sterilized nutrient broth. The tubes were incubated at 37°C for 24 hours. After incubation, each broth in a test tube was properly agitated and a loopful was inoculated on sterile salmonella-shigella agar, macConkey and eosine methylene blue agar plates by streaking method. The plates were incubated at 37°C for 24 hours, then suspected colonies of *Salmonella* and *E. coli* species were aseptically transferred to freshly prepared salmonella-shigella agar and eosine methylene blue agar plates. The plates were also incubated at 37°C for 18-24 hours. After that, pure colonies of the *Salmonella* species and *E. coli* were transferred into nutrient agar slants and were stored in the refrigerator for further biochemical identification.

### **Gram staining**

Gram staining was carried out on smears prepared from the isolates in different culture plates. The smear was prepared on clean grease-free glass slides. Each slide was allowed to air-dry and heat fixed by passing it 3 times over a busnen burner flame. The heat-fixed smear was flooded with crystal violet and allowed to stand for 30-60 seconds. The stain was washed with slowly running tap water. The smear was flooded again with Lugol's iodine and allowed to stand for 30-60 seconds. The slide was then rinsed off with slowly running tap water. The smear was decolorized with 70% ethanol until the color of the crystal violet stops coming out and was also washed off with slowly running tap water. The smear was counter-stained with safranin and allowed to stand for 30-60 seconds, washed off with slowly running tap water. The slide was then blotted dry. The slide was then examined under an oil immersion objective lens of a compound microscope. Organisms that retained the purple colour of crystal violet were recorded as Gram-positive, while those that appeared pink were Gram-negative [13].

### **Biochemical characterization of the isolates**

Conventional biochemical tests including catalase test, oxidase test, indole test, methyl red test, Voges-Proskauer test and citrate utilization were carried out on the bacterial isolates for further identification.

### **Antibiotic Sensitivity Testing**

The susceptibility and resistance pattern of the bacteria isolates to different antibiotics was determined using Kirby-Bauer disk-diffusion technique. Sterile petri – dishes of Mueller-Hinton agar was inoculated using a young culture of a 0.5 MacFarland turbidity standard of the test organism. Commercially available antibiotics discs including: gentamycin (CN) 5µg, ciprofloxacin (CIP) 30µg, ofloxacin (OFX) 10µg, chloramphenicol (C) 15 µg, colistin (CL) 5µg, nitrofurantoin (F) 30µg, chloramphenicol (C) (10µg), nitrofurantoin (N) 10 µg,

sulphamethoxazole trimethoprim (SXT) 25 µg and azithromycin (AZ) 10µg, (Oxoid, UK), were aseptically placed onto the surfaces of the inoculated plates using a sterile forceps. The plates were subsequently allowed to stand for 10-15 min to enable the inoculated organisms to pre-diffuse. The plates were incubated at 37 °C for 18-24 hrs and their clear zones of inhibition were measured to the nearest millimeters (mm) using a meter ruler. The interpretation of the measurement as sensitive or resistant were made according to the Clinical Laboratory Standard Institute interpretation guideline for antibiotics sensitivity [14].

### RESULTS

#### Percentage prevalence of *Salmonella* species and *Escherichia coli* isolated from freshly sliced pineapple sold in Abakaliki metropolis

Result obtained from this study showed that out of the 60 samples analyzed, only 12(20%) harbored *Escherichia coli* while 42(70) harbored *Salmonella* species (Table 1).

**Table 1: Percentage prevalence of *Salmonella* species and *Escherichia coli* isolated from freshly sliced pineapple sold in Abakaliki metropolis.**

Samples analyzed	Number of samples analyzed	No and % <i>Salmonella</i> species obtained	No and % <i>E. coli</i> obtained
Sliced pineapple	60	12(20)	42(70)

#### Antibiotic susceptibility and resistance pattern of *Salmonella* species and *Escherichia coli* isolated from sliced pineapple sold in Abakaliki metropolis.

In this study, the 12 isolates of *Salmonella* species screened were 100% susceptible to gentamicin, ciprofloxacin and amoxicillin and 50% susceptible each to chloramphenicol, ofloxacin, ceftriaxone and streptomycin. There were 100% resistant to nitrofurantoin, meropenem, pefloxacin, ceftriaxone (Table 2). Also, of the 42 isolates of *E. coli* screened, only 30(71.4%) were susceptible to ciprofloxacin. This was followed by 24(57.1%) each to chloramphenicol and ofloxacin, 12(28.6%) to gentamicin and 6(14.3%) each to pefloxacin, amoxicillin and streptomycin. However, there were 100% resistant to nitrofurantoin, meropenem and ceftriaxone. This high resistance was followed by 36(85.7%) each to pefloxacin, amoxicillin and streptomycin and 30(71.4%) to ciprofloxacin (Table 3).

*E. coli* species

**Table 2: Antibiotic susceptibility and resistance of *Salmonella* species isolated from sliced pineapple sold in Abakaliki metropolis.**

Antibiotics used	number and % susceptible	number and % resistant
Nitrofurantoin	0(0)	12(100)
Gentamicin	12(100)	0(0)
Ciprofloxacin	12(100)	0(0)
Chloramphenicol	6(50)	6(50)
Ofloxacin	6(50)	6(50)
Meropenem	0(0)	12(100)
Pefloxacin	0(0)	12(100)
Ceftriaxone	0(0)	12(100)
Amoxycillin	12(100)	6(50)
Streptomycin	6(50)	6(50)

**Table 4. Antibiotic susceptibility and resistance pattern of *Escherichia coli* isolated from freshly sliced pineapple sold in Abakaliki metropolis.**

Antibiotic resistance	number and % susceptible	number and % resistant
Nitrofurantoin	0(0)	42(100)
Gentamicin	12(28.6)	30(71.4)
Ciprofloxacin	30(71.4)	12(28.6)
Chloramphenicol	24(57.1)	18(42.9)
Ofloxacin	24(57.1)	18(42.9)
Meropenem	0(0)	42(100)
Pefloxacin	6(14.3)	36(85.7)
Ceftriaxone	0(0)	42(100)
Amoxicillin	6(14.3)	36(85.7)
Streptomycin	6(14.3)	36(85.7)

### DISCUSSION

Fruits generally are very important food resources because of their high dietary and nutritional values. Vending of sliced fruits like pineapple, watermelon and pawpaw has become a lucrative business in Nigeria because of the high patronage it attracts. However, poor handling of the sliced fruit may make it an important source of infections. This study assessed the occurrence of *Salmonella species* and *E. coli* in sliced pineapple sold in Abakaliki Metropolis in Nigeria. The result revealed very high occurrence of *E. coli* (70%) and a relatively low occurrence of *Salmonella species* (20%). The presence and high prevalence of *E. coli* in this study signals recent contamination by fecal matter and possible presence of other enteric pathogens in the fruits that can cause food borne gastroenteritis and bacterial diarrhea [20, 27]. Presence of these organisms in fruits can also be associated with the nutritional composition and high water availability in fruits, which encourage the growth and survival of these microorganisms [28-30]. The high level of contamination also could be linked to the level of exposure and the handling of the fruits. It has been observed that most of the vended fruits and stores are situated very close to major roads where passersby can easily have access to them. This exposes the fruits to dust and other contaminants [7]. Poor handling by vendors together with the unhygienic market environments are factors that contribute to the high microbial load in the ready-to eat fruits [10-13]. The common practice of using same bucket of water to wash all the fruits if it is ever washed at all and the use of same utensils such as knife for cutting may also be contributing immensely to the high microbial loads of the sliced fruits [15-17]. Pathogens may invade the interior of the product while carrying out the preparatory processes such as peeling, slicing, trimming and marketing [18-21]. Meanwhile, the chances of contamination is intensified by the fact that ready-to-eat sliced fruit vending is practiced without adequate storage conditions, thereby exposing the sliced fruits to flies and high temperature of the open environment which may encourage invasion and proliferation of contaminants [24]. Our findings agrees with the study by [17-19] 2022 who isolated *E. coli* and *Salmonella species* from sliced pineapple fruits sold at Apepe Sango and University of Ibadan in Oyo State, and in Port Harcourt Rivers State, respectively, both in Nigeria with *E. coli* being more prevalent than *Salmonella species*. Also similar to our findings, [17] reported presence of *Salmonella spp.* (12.5%) and *E. coli* (4.2%) in ready to eat fruits and vegetable in Sango-Ota,

Ogun State Nigeria. [10], also reported the presence of enteric pathogens including *E. coli* and *Salmonella* spp. in ready-to-eat fruits. The presence of these enteric pathogens is of great public concern because of their implication in food-related outbreaks. Several cases of typhoid fever outbreak have been associated with eating contaminated vegetables grown in or fertilized with contaminated soil or sewage [3]. Antibiotic resistance has become a worldwide menace which has led to the increased rate of morbidity and mortality as a result of infections caused by resistant microorganisms. [6], also reported that *E. coli* isolated from foods were sensitive to Streptomycin, cotrimoxazole and ciprofloxacin. [9], reported that *Escherichia* species and *Klebsiella* species isolated from vegetables showed high sensitivity to Amoxicillin/clavulanic acid and that 12% of *E. coli* isolated from vegetable were resistance to chloramphenicol.

The antibiotic susceptibility and resistance pattern of the *Salmonella* species and *E. coli* species obtained in this study varied. *Salmonella* species were totally susceptible to gentamycin, ciprofloxacin and amoxicillin; 50% susceptible to chloramphenicol, ofloxacin, streptomycin. There were 100% resistant to nitrofurantoin, meropenem, pefloxacin and ceftriaxone. Also, *E. coli* showed highest susceptibility to ciprofloxacin, (71.4%), chloramphenicol and ofloxacin, (57.1% each). This study showed that the *Salmonella* species and *E. coli* isolated from sliced pineapple are multidrug resistant. Their resistance to other antibiotics used ranged from 28.6% -100%. The presence of resistant bacteria pathogen may be linked to continuous and indiscriminate use of antibiotics for therapeutic purpose and as an additive [3]. Abuse of antibiotics have resulted in increase in prevalence of antibiotic resistant bacteria strains, vice-verse increase bacterial infections [8]. Resistance could as well be as a result of presence of some drug resistant genes, additional gain of other genes through horizontal gene transfer or by physiology dependent resistance [10]. This finding corroborates with the study of [11] who also recorded greater percentage of susceptibility of the isolates obtained from sliced fruits to gentamicin, ofloxacin and ciprofloxacin and least to nitrofurantoin. This study and other studies by researchers have demonstrated that pathogens are present in ripe fruits and the microorganisms are multi-drug resistant which of public health concern.

### CONCLUSION

This study revealed that the growing practice of selling sliced fruits, including pineapple, in Abakaliki and other parts of Nigeria, is contributing significantly to the spread of infectious diseases in the region and the spread of multi-drug resistant pathogens in particular. It points to the unhygienic handling of the sliced fruits vending and calls for appropriate action by the relevant health authorities to minimize spread of diseases through this route.

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